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RESEARCH ARTICLE | *Control of Movement*

Time course of functional recovery during the first 3 mo after surgical transection and repair of nerves to the feline soleus and lateral gastrocnemius muscles

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Gregor RJ, Maas H, Bulgakova MA, Oliver A, English AW, Prilutsky BI. Time course of functional recovery during the first 3 mo after surgical transection and repair of nerves to the feline soleus and lateral gastrocnemius muscles. *J Neurophysiol* 119: 1166–1185, 2018. First published November 29, 2017; doi:10.1152/jn.00661.2017.—Locomotion outcomes after peripheral nerve injury and repair in cats have been described in the literature for the period immediately following the injury (muscle denervation period) and then again for an ensuing period of long-term recovery (at 3 mo and longer) resulting in muscle self-reinnervation. Little is known about the changes in muscle activity and walking mechanics during midrecovery, i.e., the early reinnervation period that takes place between 5 and 10 wk of recovery. Here, we investigated hindlimb mechanics and electromyogram (EMG) activity of ankle extensors in six cats during level and slope walking before and every 2 wk thereafter in a 14-wk period of recovery after the soleus (SO) and lateral gastrocnemius (LG) muscle nerves in one hindlimb were surgically transected and repaired. We found that the continued increase in SO and LG EMG magnitudes and corresponding changes in hindlimb mechanics coincided with the formation of neuromuscular synapses revealed in muscle biopsies. Throughout the recovery period, EMG magnitude of SO and LG during the stance phase and the duration of the stance-related activity were load dependent, similar to those in the intact synergistic medial gastrocnemius and plantaris. These results and the fact that EMG activity of ankle extensors and locomotor mechanics during level and upslope walking recovered 14 wk after nerve transection and repair suggest that loss of the stretch reflex in self-reinnervated muscles may be compensated by the recovered force-dependent feedback in self-reinnervated muscles, by increased central drive, and by increased gain in intermuscular motion-dependent pathways from intact ankle extensors.

NEW & NOTEWORTHY This study provides new evidence that the timeline for functional recovery of gait after peripheral nerve injury and repair is consistent with the time required for neuromuscular junctions to form and muscles to reach preoperative tensions. Our findings suggest that a permanent loss of autogenic stretch reflex in self-reinnervated muscles may be compensated by recovered intermuscular force-dependent and oligosynaptic length-dependent feed-

back and central drive to regain adequate locomotor output capabilities during level and upslope walking.

cat; muscle length and force feedback; muscle self-reinnervation; peripheral nerve injury; proprioceptive feedback; slope walking; spinal reflexes

INTRODUCTION

The time course of functional recovery after surgical transection and repair of peripheral nerves in the cat hindlimb involves a continuum of interdependent changes in the size and fiber composition of the target muscles and their functional synergists (Bodine-Fowler et al. 1993; Gordon and Stein 1982; Rafuse and Gordon 1996a, 1996b; Unguez et al. 1996), as well as changes in central drive and motion-dependent afferent inputs regulating task-specific motor output (Donelan et al. 2009; Frigon and Rossignol 2008b; Gritsenko et al. 2001; Maas et al. 2010; Pearson and Misiasek 2000). The immediate effects of nerve transection and repair, i.e., the initial 3–4 wk of recovery, include a well-documented period of paralysis in the target muscles and a significant compensatory increase in electromyogram (EMG) output in their intact synergists (Frigon and Rossignol 2008a; Maas et al. 2010; Mehta et al. 2015; Pearson et al. 1999; Prilutsky et al. 2011). Motor output patterns during this period show compensatory changes in hindlimb posture and gait kinematics affecting muscle-tendon unit and fascicle length changes during level and slope walking. These changes allow for the preservation of hindlimb length, joint kinetics, and ground reaction forces during slope walking (Abelew et al. 2000; Chang et al. 2009; Maas et al. 2010; Mehta et al. 2015; Prilutsky et al. 2011; Sabatier et al. 2011). These findings are relevant to our understanding of the significance of the observed compensations, their functional consequences, and their potential for informing therapy interventions during the acute phase of recovery after nerve transection and repair (Amado et al. 2011).

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Surgical transection and repair of peripheral nerves result in the permanent loss of autogenic muscle stretch-dependent Ia-afferent feedback in the self-reinnervated muscle (Alvarez et al. 2011; Bullinger et al. 2011; Cope and Clark 1993; Cope et al. 1994; Haftel et al. 2005; Maas et al. 2007). The long-term significance of this loss in ankle extensors, discussed in the context of achieving relatively normal locomotor output, lies in the fact that gait kinematics and kinetics appear to be quite similar to control values after a recovery period of ~11–14 wk, except during downslope walking when joint angle patterns do not return to control values (Abelew et al. 2000; Gregor et al. 2014; Maas et al. 2007; Pantall et al. 2016). Maas et al. (2007), for example, followed the recovery period of self-reinnervation of the cat medial and lateral gastrocnemius muscles reporting that an exaggerated ankle yield in the stance phase of downslope walking persisted for 57 wk.

As reviewed above, there are substantive data on the compensatory locomotor changes in response to peripheral nerve transection and repair in the first 3 wk before any signs of muscle reinnervation have been reported. Similarly, long-term effects (3 mo and beyond) of nerve transection and repair with subsequent muscle self-reinnervation are well documented. What has been missing in the literature, however, is information on muscle activity and locomotor mechanics during the period between the first appearance of muscle activity (the early reinnervation phase, 5–6 wk; Gordon and Stein 1982; Gregor et al. 2014) and the time when the magnitude of muscle activity approaches pre-nerve transection values, 10–12 wk (Pantall et al. 2016). This information is critical to the design of specific rehabilitation interventions targeting either compensatory strategies and promotion of nerve regeneration before any muscle reinnervation occurs or the reinnervating muscles affecting recovering muscle motoneuronal output and/or sensory feedback (Boeltz et al. 2013; Brandt et al. 2015).

Because little is known about the changes in muscle activity and walking mechanics during the critical first 3 mo after nerve transection and repair, the goal of this study was to describe the changes in EMG timing and magnitude in the reinnervated lateral gastrocnemius (LG) and soleus (SO) muscles and their intact synergists medial gastrocnemius (MG) and plantaris (PL) muscles, as well as the changes in hindlimb walking mechanics every 2 wk during the initial 14-wk period of recovery. We hypothesized that significant changes would occur in locomotor EMG activity and hindlimb locomotor

mechanics during the early reinnervation period and that these changes would be consistent with the time frame of recovery in the neuromuscular self-reinnervation process (Gordon and Stein 1982).

METHODS

The six cats used in these series of experiments (see Table 1 for body mass) were the same as those described in a previous report (Pantall et al. 2016). All surgical and experimental procedures were similar to those previously reported (Gregor et al. 2006; Maas et al. 2007; Pantall et al. 2016; Prilutsky et al. 2011), and all were conducted in agreement with the *Principles of Laboratory Animal Care* (National Institutes of Health publication no. 86-23, revised 1985) and approved by the Georgia Tech Institutional Animal Care and Use Committee.

Experimental Procedures

A brief description of the experimental procedures and data analysis is provided below. Initially, each cat was trained using food reward to walk at a self-selected speed along a custom-made Plexiglas-enclosed walkway with three force plates (Bertec, Columbus, OH) concealed in the walkway floor. Three separate walking conditions were tested: downslope (−50%, −27°), level (0%, 0°), and upslope (+50%, +27°).

Two separate surgeries were performed on each cat under aseptic conditions using isoflurane anesthesia. During the first survival surgery, bipolar EMG electrodes were implanted in the midbellies of the right SO, MG, and LG muscles in each of the six cats (*cats BL, CO, GE, IN, KO, and NA*, Table 1) and in the distal portion of the PL in two cats (*cats BL and CO*). Pain medication and antibiotics were administered after the surgery, and the animals recovered for 10–14 days. Subsequently, EMG activity, hindlimb kinematics, and ground reaction forces were recorded in all three walking conditions for several weeks to obtain baseline data.

A second survival surgery was conducted in all cats to transect and immediately reconnect, using fibrin glue, the branch of the tibial nerve innervating the SO and LG muscles. After 3–5 days of postoperative recovery, locomotion data collection continued weekly or biweekly until week 14 after surgical nerve transection and repair.

In addition, surgical biopsies were taken under isoflurane anesthesia from the self-reinnervated SO and LG muscles of *cats BL and CO* and the self-reinnervated MG muscle of two cats from a previous study (Maas et al. 2007) at different times (5–108 days) after nerve transection and repair (Table 2). The samples of muscle removed were fixed by immersion in 4% paraformaldehyde for 1 h and then stored in a cryoprotection solution of 20% sucrose for at least 24 h before being sectioned for histology.

Table 1. *Body and muscle mass*

Cat	Time After Surgery, wk	Body Mass, kg	Muscle Mass of Affected Hindlimb, g				Muscle Mass of Intact Hindlimb, g			
			SO	LG	MG	PL	SO	LG	MG	PL
<i>BL</i>	16	3.30	4.28	8.42	11.95	7.23	4.39	11.37	10.65	6.95
<i>CO</i>	15	4.3	6.11	12.36	14.56	9.47	6.58	15.53	14.16	8.41
<i>GE</i>	49	4.75	4.30	16.29	15.79	9.01	4.32	18.62	15.29	8.13
<i>IN</i>	30	3.35	4.77	11.86	12.46		4.27	13.15	11.94	
<i>KO</i>	19	3.15	2.94	10.13	10.87		2.9	8.70	11.25	
<i>NA</i>	36	3.23	6.22	15.40	8.75		4.12	6.77	12.88	
Mean ± SD	27.5 ± 13.4	3.68 ± 0.67	4.77 ± 1.24	12.14 ± 3.01	12.40 ± 2.53	8.57 ± 1.18	4.43 ± 1.19	12.36 ± 4.37	12.70 ± 1.78	7.83 ± 0.77

SO, soleus; LG, lateral gastrocnemius; MG, medial gastrocnemius; PL, plantaris. The effects of self-reinnervation of the SO and LG muscles on any differences in muscle mass between the contralateral, i.e., intact nonsurgical, hindlimb and the affected (surgical) hindlimb were not significant: SO ($F_{1,29} = 0.091$, $P = 0.765$), LG ($F_{1,29} = 0.002$, $P = 0.963$), MG ($F_{1,29} = 0.070$, $P = 0.793$), and PL ($F_{1,29} = 0.216$, $P = 0.646$).

Table 2. Number of synapses identified in muscle biopsies

Cat	Muscle	Days after Nerve Transaction and Repair	Number of Synapses	Percentage of Occupied Motor Endplates
BL	SO	55	45	84.4
	LG	55	20	100
	LG	108		100
CO	SO	104	24	79.0
	LG	104	20	100
LS	MG	5	0	0
		75	18	72.2
QL	MG	25	41	22.0
		75	28	100

Cats LS and QL are from a previous study (Maas et al. 2007) in which MG and LG muscles were self-reinnervated.

During locomotion experiments, three-dimensional positions of small reflective markers on the right hindlimb anatomical landmarks were recorded by a six-camera motion capture system (Vicon Motion Systems, Oxford, United Kingdom) at a sampling rate of 120 Hz. Ground reaction forces and EMG activity from each muscle were recorded at 360 and 3,000 Hz, respectively. All recordings were synchronized by a common pulse. During each session, 30–60 trials of walking were recorded, and only trials during which the cat moved with a steady speed and contacted at least 1 force plate with the right hindlimb were selected for further analysis. To minimize the effects of walking speed on the outcome variables and yet obtain an appropriate number of trials for analysis, trials with a stance time between 400 and 650 ms were used for analysis.

At the conclusion of the experimental sessions for each individual cat (15–49 wk after nerve transection and repair, Table 1) a terminal experiment was conducted in the decerebrate state to measure stretch reflexes and determine the mass of SO, LG, MG, and PL muscles in both hindlimbs. The terminal experiments have been described in detail previously (Lyle et al. 2016; Maas et al. 2007). After completion of the necessary measurements, the cat was euthanized by either an overdose of concentrated pentobarbital (Euthasol) or potassium chloride. Potassium chloride was used under isoflurane anesthesia.

Data Analysis

To study the effect of time and slope on the recovery of gait mechanics and locomotor EMG activity after peripheral nerve transection and repair, data were divided into successive 2-wk periods of time resulting in seven separate analysis periods: control (C), i.e., pre-nerve transection and repair, and weeks 1–2 (W1–2), W3–4, W5–6, W7–8, W9–10, and W11–14 of recovery. The last analysis period was extended by 2 wk to collect additional trials for statistical analysis. This presentation of functional outcome measures provided more data points during the recovery period than in previous reports (Maas et al. 2007, 2010; Mehta and Prilutsky 2014; Mehta et al. 2015; Pantall et al. 2016; Prilutsky et al. 2011).

Computed joint angles were time normalized to the duration of stance and swing time separately. Time-normalized joint angle patterns were averaged at each percentage of the normalized time across cycles of each cat and slope and then across cats for each slope. The joint yield in stance was calculated for the ankle and knee joints in each cycle as the difference between the angle at stance onset (paw contact) and the minimum angle in stance.

Resultant joint moments were computed for the hip, knee, and ankle using recorded hindlimb kinematics, ground reaction force components orthogonal to the walking surface (normal component) and parallel to the walking surface (tangential component), and principles of inverse dynamics (Fowler et al. 1993; Gregor et al. 2006;

Prilutsky et al. 2011). Time-dependent ground reaction forces and joint moments were time normalized to the duration of stance and swing phases separately. The magnitude of the ground reaction forces and joint moments were scaled to body mass. Analysis of resultant joint moments focused on the peaks of extension and flexion moments at the hip, knee, and ankle during the stance phase of walking at three separate slope conditions.

Bursts of EMG activity from each muscle (EMG_b) were identified on the basis of a threshold of 2 SD above baseline of full-wave rectified EMG activity. Exemplar raw EMG burst patterns for the SO, LG, MG, and PL muscles at each slope for control (C), W1–2, and W11–14 are presented in Fig. 1A. Mean magnitude and duration of three separate phases of rectified EMG_b activity were calculated for each muscle. The three phases included precontact EMG activity (burst onset to paw contact, EMG_{pre}), stance EMG activity (paw contact to EMG offset, EMG_{st}), and total burst EMG activity (EMG onset to offset, EMG_b ; Fig. 1B). Note that the EMG_{pre} activity primarily reflects the central input to motoneurons (Gritsenko et al. 2001; Pearson et al. 1999) and EMG_{st} activity is related to both the central drive and muscle stretch and limb load reflex inputs induced by contact with the ground. The burst EMG_b activity is the sum of EMG_{pre} and EMG_{st} and reflects the overall muscle activity during the walking cycle. Mean EMG magnitude for each muscle, calculated as the EMG time integral/EMG duration for each of the three phases studied, was scaled to the maximum mean magnitude of the total EMG burst (EMG_b) obtained in W11–14 within each cat and muscle across all walking conditions. EMG activity data were analyzed, and group means were calculated across 5–63 cycles per muscle, per condition, per time period for all 6 cats.

Fixed and cryoprotected muscle biopsies were frozen and mounted on the chuck of a cryostat for sectioning. Care was taken in mounting the samples so that sectioning took place in a longitudinal plane with respect to the orientation of muscle fibers in the sample. Sections were made at a thickness of 40 μ m and mounted directly onto cold, subbed slides. They were reacted with antibodies to the highest-molecular weight neurofilament protein (NF-H, N4142; Sigma) and the synaptic vesicle protein, SV2 (Developmental Studies Hybridoma Bank), followed by species-appropriate secondary antibodies and α -bungarotoxin conjugated to Alexa Fluor 555 (B35451; Thermo Fisher). Motor endplates in these sections, identified by binding fluorescent bungarotoxin, served as markers of sites where neuromuscular synapses will be re-formed. Immunoreactivity to NF-H marks the cytoskeleton of the axon and was used here to detect the presence of regenerating axons at motor endplates. We interpreted the NF-H staining in the synaptic terminal, not just the axon cylinder, as evidence that the terminal was not just formed but had been in place for a while. SV2 immunofluorescence was used to indicate when synaptic vesicles had returned to reinnervated endplates and marked the presence of a re-formed synapse. On average, 20 endplates from each muscle sample studied were scored. Endplates were located by scanning the lengths of muscle fibers in sections at low magnification ($\times 10$) but were studied at $\times 40$ magnification. They were selected for study only if they were seen in their entirety, or en face. Endplates that were visualized only on their sides were encountered often, but they were not studied. Endplates were selected for study in several sections through each biopsy in an effort to reduce or eliminate bias or differences in the regions of the biopsies sampled. No effort was made to quantify the extent to which the endplate was reoccupied by an overlying synapse. An endplate was scored as not innervated if no immunoreactivity to either molecule was present.

Statistical Analysis

A linear mixed-effects (MIXED; SPSS 19.0; SPSS, Chicago, IL) model was used to test the effects of the independent fixed

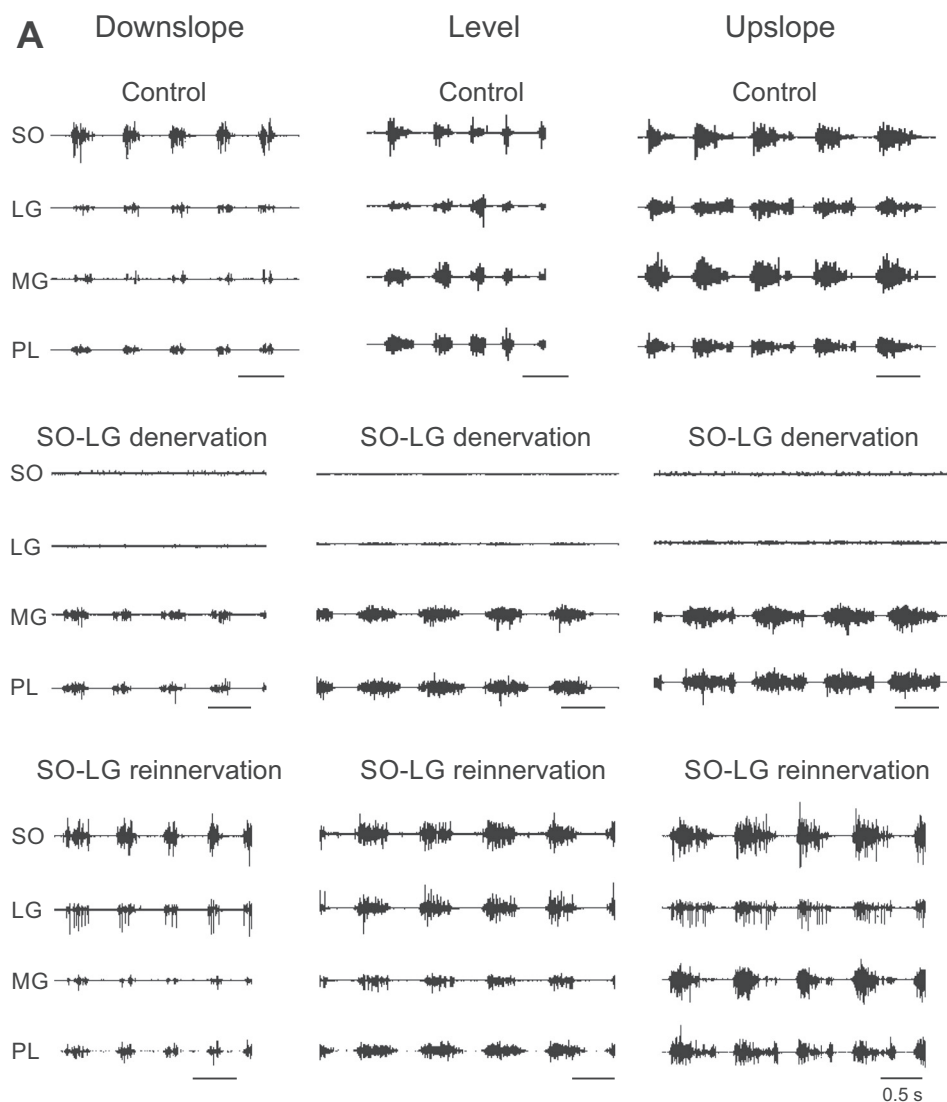
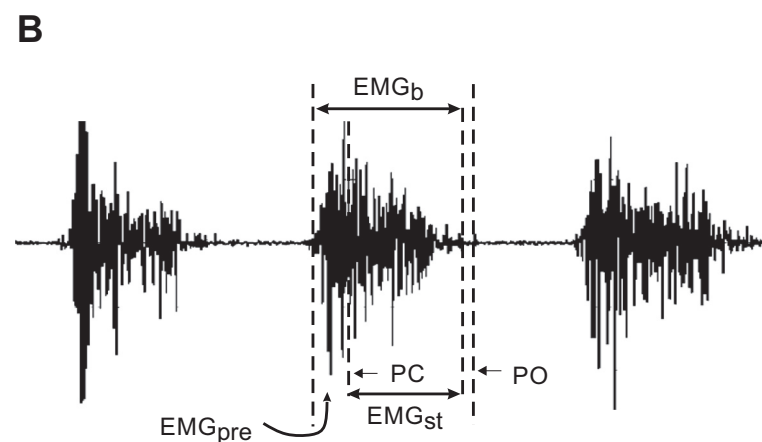


Fig. 1. Examples of raw electromyograms (EMG) of ankle extensors during walking in cat CO. A: EMG recorded from ankle extensors during downslope, level, and upslope walking before transection and repair of soleus and medial gastrocnemius nerves (control), SO-LG denervation 1–2 wk after surgery (W1–2), and SO-LG reinnervation 11–14 wk after surgery (W11–14). B: division of an EMG burst into three phases: precontact EMG (EMG_{pre}), EMG activity during stance (EMG_{st}), and EMG total burst (EMG_b). SO, soleus; LG, lateral gastrocnemius; MG, medial gastrocnemius; PL, plantaris; PC, paw contact; PO, paw liftoff.



factors, time (7 levels comprising control and six 2-wk time periods during recovery) and slope (3 levels), on each dependent variable. The dependent variables included six EMG outcome measures (duration and magnitude of precontact burst, stance burst, and total burst, Fig. 1B), peak extension and flexion resultant moments at the ankle, knee, and hip joints during the stance phase of walking, as well as the magnitude of joint yield for the ankle and knee joints across recovery time. In this analysis, cats and cycles

were considered random factors. Stance duration was used as a covariate to account for potential effects of walking speed. To determine effects of self-reinnervation and muscle (fixed factors) on muscle mass measured in the terminal experiment (dependent variable), a similar linear mixed-effects model analysis was employed. When a significant difference was identified ($P < 0.05$), a post hoc paired comparison with Bonferroni adjustment was performed.

RESULTS

Muscle Mass and Formation of Neuromuscular Synapses

Muscle mass in the affected hindlimb measured in the terminal experiments 15–49 wk after SO-LG nerve transection and repair was not significantly different from the corresponding muscles in the intact limb ($F_{1,29} = 0.002\text{--}0.216$, $P = 0.646\text{--}0.963$, Table 1). For example, masses of self-reinnervated and contralateral SO were 4.77 ± 1.24 and 4.43 ± 1.19 g (means \pm SD), respectively; the corresponding values for LG were 12.14 ± 3.01 and 12.36 ± 4.37 g, respectively. The terminal experiments confirmed the absence of autogenic stretch reflex in the self-reinnervated SO and LG [these results have been described previously (Lyle et al. 2016) and are not shown here].

To assess the effect of time on synapse development and where synapses were formed (Gordon and Stein 1982; Sanes and Lichtman 1999), biopsies taken from self-reinnervated muscles of four cats at different time points in recovery were analyzed (Table 2 and Fig. 2). In *cat BL*, the SO muscle had 84% occupation of motor endplates and the LG had 100% occupation at 55 days (W7–8), while in *cat CO* the SO muscle

had 79% occupation and the LG had 100% occupation at 104 days (15 wk). Following MG/LG self-reinnervation, a biopsy of the MG was obtained in two separate cats (*cats LS* and *QL*) showing 72 and 100% occupation, respectively, at 75 days (W11–14). No motor endplates were occupied in the self-reinnervated MG at 5 days (W1–2, 0%, *cat LS*) while a small percentage were occupied at 25 days (W3–4, 22%, *cat QL*); see Table 2.

Walking Mechanics

Ground reaction forces. Peak values of the normal component, i.e., component orthogonal to the walking surface, of ground reaction forces (Fig. 3) were affected by time ($F_{6,774} = 33.9$, $P < 0.001$) and slope ($F_{2,774} = 1,186.0$, $P < 0.001$) after nerve transection and repair. The peak normal force component increased from downslope to level and to upslope walking conditions (Fig. 3). A decrease in the peak normal force from control values (C) occurred only during downslope walking at time windows W1–2 through W5–6 ($P < 0.001$); peak normal force returned to control values at W7–8 ($P = 0.099$, Fig. 3B). No force decrease below the presurgery levels was observed during level or upslope walking (Fig. 3B); during level and

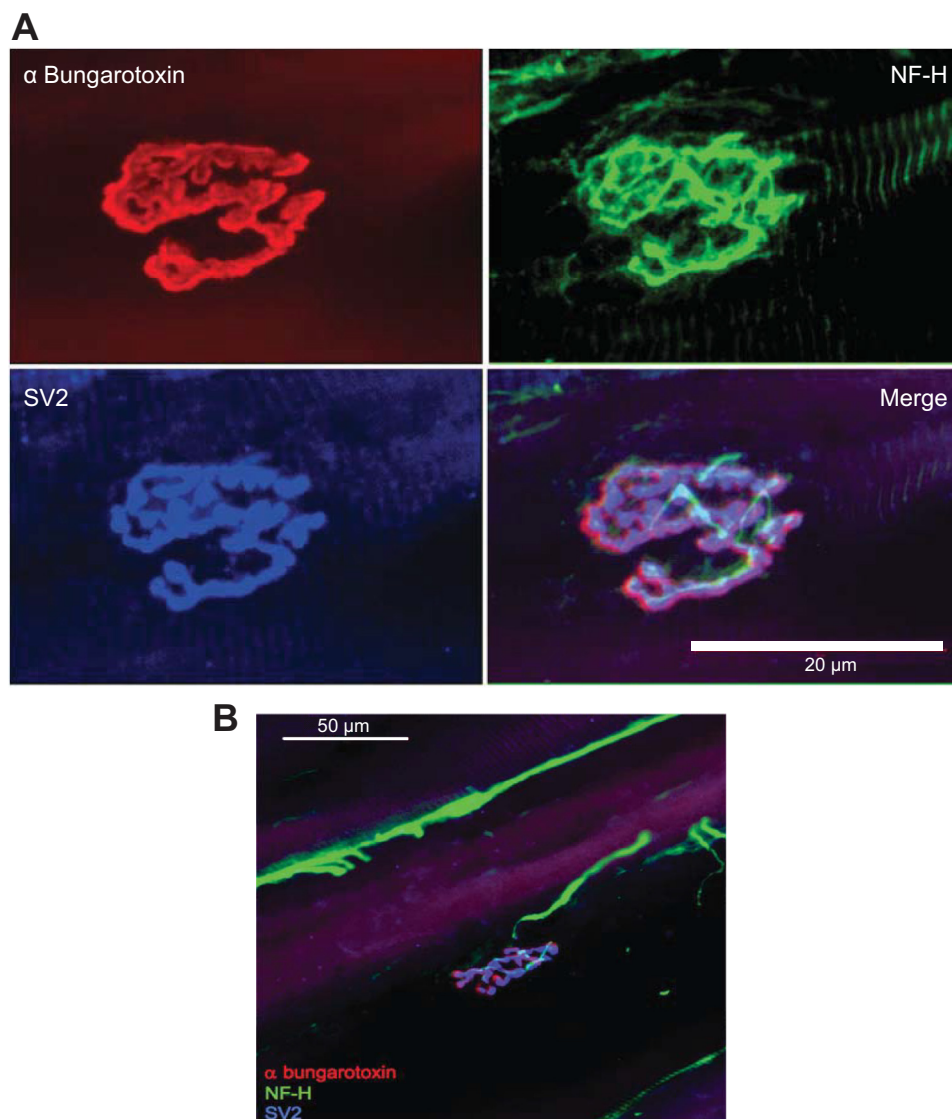


Fig. 2. Examples of images obtained from muscle biopsies. *A*: images are from lateral gastrocnemius (LG) of *cat BL* 8 wk after transection and repair of the LG-soleus (SO) nerve. All endplates studied in this cat were reoccupied. (Scale bar = 20 μ m.) *B*: synaptic terminal in the LG in *cat BL* 55 days after LG-SO nerve transection and repair. α -Bungarotoxin is used as a marker for synapse location, highest-molecular weight neurofilament protein (NF-H) marks the cytoskeleton of the axon, and synaptic vesicle protein-2 (SV2) is a marker for the return of synaptic vesicles to reinnervated endplates. All endplates studied in the cat were reoccupied. (Scale bar = 50 μ m. See further details in the text.)

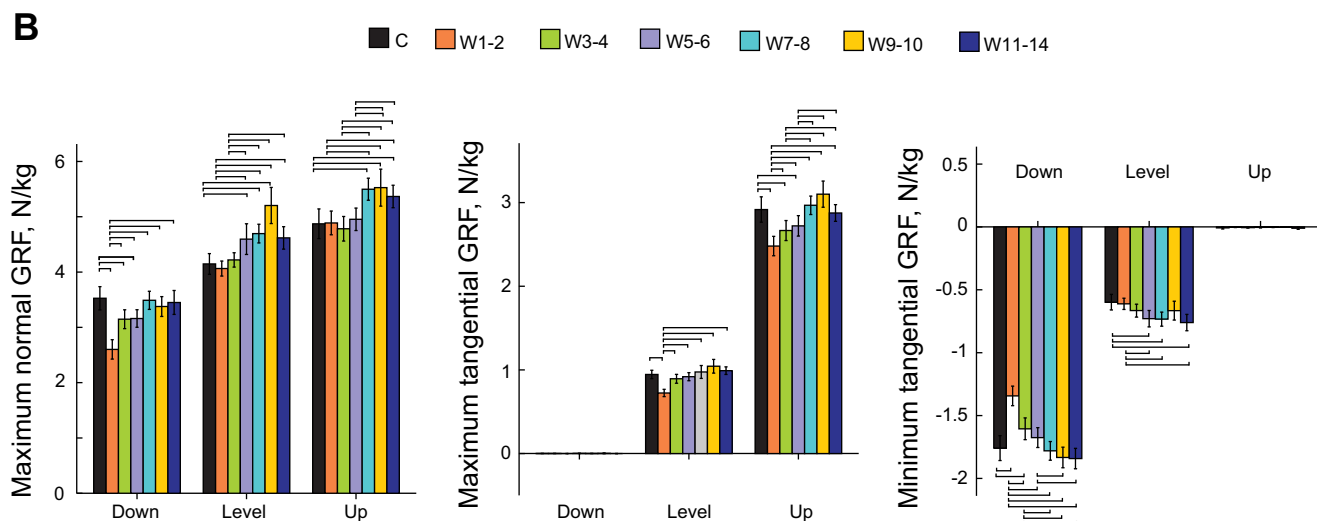
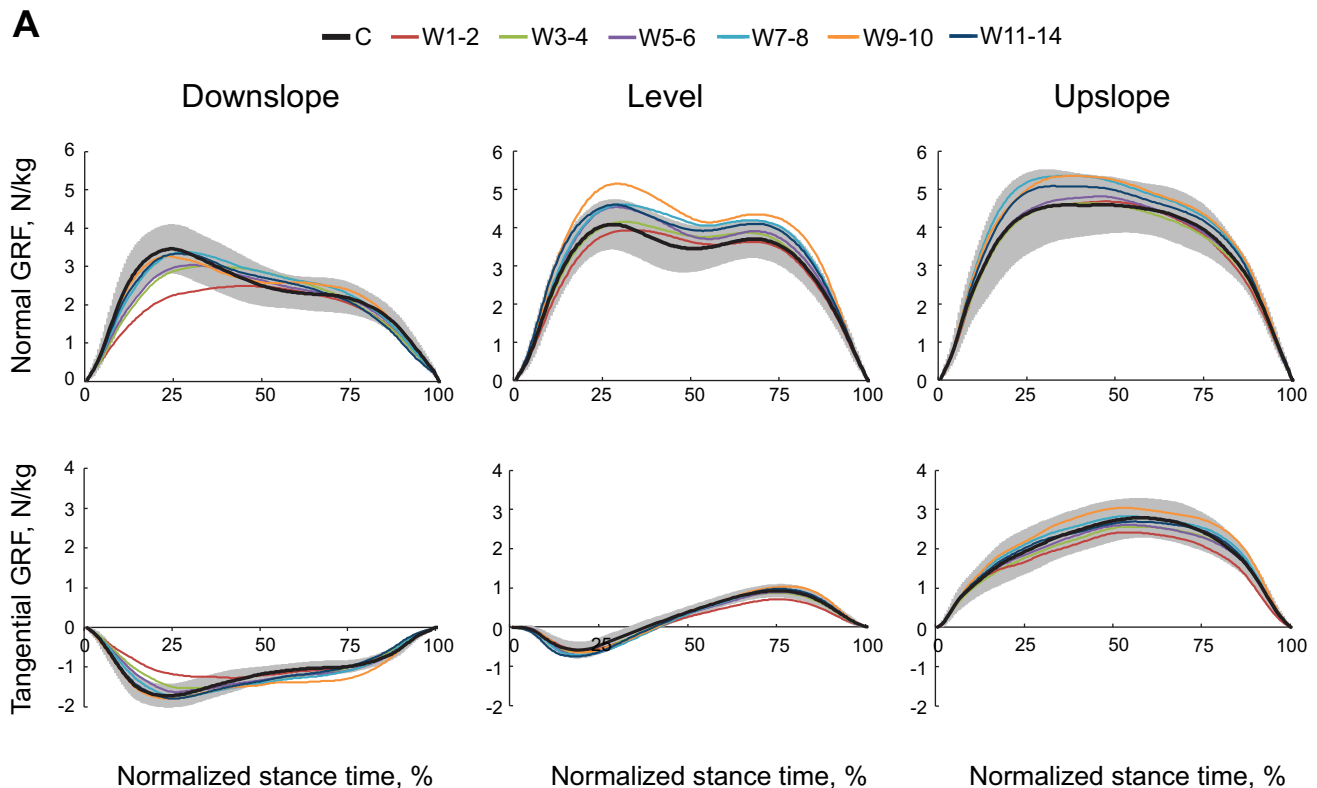


Fig. 3. Normal and tangential components of ground reaction forces (GRF) during walking. Control (C), before transection and repair; W1–2, W3–4, W5–6, W7–8, W9–10, and W11–14 are successive 2-wk periods of analysis after nerve transection and repair. A: patterns of the normal and tangential components of GRF during downslope, level, and upslope walking. The thick black lines and gray shadows indicate the mean force and SD, respectively, obtained in control. The thin colored lines are the mean forces at different times of recovery (W1–2 through W11–14). B: positive and negative peaks (means \pm 95% confidence interval) of normal and tangential GRF before Control and at 2-wk intervals after nerve transection and repair (W1–2 through W11–14). The horizontal lines between the vertical bars indicate statistical significance ($P < 0.05$).

upslope walking, contrary to downslope walking, the peak normal force increased above the baseline values at W5–6 through W11–14 (Fig. 3B).

Tangential ground reaction forces were negative during downslope walking, positive during upslope walking, and changed sign from negative to positive at $\sim 35\%$ of the stance phase during level walking (Fig. 3A). Correspondingly, the

maximum and minimum force values were higher and lower compared with those in level walking during upslope and downslope walking, respectively ($F_{2,774} = 8,940.4$, $P < 0.001$). The maximum (positive peak) values were lower than control values (C) at W1–2 for level walking and at W1–2 through W5–6 for upslope walking ($P < 0.001$) but reached the presurgery control values by W3–4 and W7–8, respec-

tively ($P < 0.050$, Fig. 3B). Minimum (negative peak) values were greater than baseline values at W7–8 through W11–14 during level walking ($P < 0.050$). During downslope walking, significantly smaller negative peak forces were produced compared with control at time windows W1–2 through W3–4 ($P < 0.001$, Fig. 3). The minimum values of tangential forces during downslope walking reached control values by time window W5–6 ($P = 0.387$); during level walking, the negative peak forces exceeded the baseline values starting at time windows W5–6 ($P < 0.006$) and W7 ($P < 0.002$; Fig. 3B). Negative tangential forces during upslope and positive tangential forces during downslope walking were negligible (Fig. 3). Thus a decrease in peaks of the normal and tangential components of ground reaction forces immediately after nerve transection and repair occurred mostly in downslope walking, and the force peaks returned to controls by W7–8.

Joint moments. Patterns and peaks of extension and flexion moments at the hip and knee joints changed with slope conditions. For instance, early in the stance phase, duration of the hip extension moments increased with slope occupying the initial ~20, ~40, and ~50% of stance during downslope, level, and upslope walking, respectively, while duration of the knee flexion moment increased from ~10% in initial stance during downslope and level walking to ~20% during upslope walking (Fig. 4A). The magnitude of the peak extension moments for the hip and knee increased from downslope to level and to upslope walking ($F_{2,3137} = 1,049.8$, $P < 0.001$). Peak flexion moments were also significantly affected by the slope ($F_{2,3139} = 31.2$, $P < 0.001$); hip flexion moments increased from the level and upslope to downslope condition, whereas knee flexion moments increased from the downslope and level to upslope condition (Fig. 4B).

Time of recovery after nerve transection and repair significantly affected peak extension ($F_{6,3138} = 12.422$, $P < 0.001$) and peak flexion ($F_{6,3135} = 4.005$, $P = 0.001$) moments. Peak extension moments across joints and slopes were significantly smaller than control in W1–2 ($P < 0.001$) but were not significantly different from control for the remainder of the 14-wk recovery period (W3–4 through W11–14, $P < 0.050$). Post hoc tests revealed that smaller extension moment peaks in W1–2 compared with control occurred only at the hip joint during upslope walking ($P < 0.001$, Fig. 4B). Peak flexion moments post-nerve transection and repair were not smaller than the control values except for the hip flexion moment during downslope walking (Fig. 4B). Thus a decrease in peaks of flexion and extension moments compared with control values occurred only at the hip joint during downslope and upslope walking, respectively, in W1–2. Joint moment peaks at other joints and in other slope conditions either did not change or slightly increased following nerve transection and repair.

Joint yield. The magnitude of joint yield at the ankle and the knee during stance was affected by time of recovery ($F_{6,2333} = 16.4$, $P < 0.001$) and slope ($F_{2,2333} = 2,628.1$, $P < 0.001$; Fig. 5), while the magnitude of joint yield at the hip was negligible (not shown) and not affected by time of recovery ($P = 0.081$) or slope ($P = 0.364$). Ankle and knee joint yield responded differently to changes in slope and time of recovery. Ankle joint yield was significantly greater than control at all slopes and weeks except during level and upslope walking in W9–10 and upslope walking in W11–14 ($P < 0.05$). There were no significant differences between ankle yield magnitudes

in successive weeks at any of the three slope conditions except for downslope walking where values in W7–8 were significantly less than those in W5–6 ($P = 0.002$) and values in W11–14 were significantly greater than those in W9–10 ($P = 0.007$; see Fig. 5).

The knee joint had significantly less yield than control in W1–2 ($P < 0.001$) and significantly greater yield in W3–4 compared with W1–2 ($P < 0.001$) during downslope walking. Also during downslope walking the magnitude of knee yield was significantly less in W7–8 compared with W5–6 ($P = 0.001$), significantly greater in W9–10 compared with W7–8 ($P < 0.001$) and control ($P < 0.001$), and significantly greater than control in W11–14 ($P = 0.003$) but less than in W9–10 ($P = 0.006$). While the magnitude of knee yield was always less than control, i.e., the knee was relatively more extended during upslope walking, there were no significant differences at any week compared with control values or between successive weeks in level and upslope walking ($P = 0.097$ – 1.0). The magnitude of hip joint yield, defined in the same way as for the ankle and knee, was close to zero and not significantly different from control (C) at any week or between successive weeks at any slope condition during recovery ($P < 0.05$).

EMG Activity

EMG activity of self-reinnervated SO and LG muscles. The mean magnitude of SO locomotor EMG activity in the three phases of activity studied (Fig. 1B) was affected by the time of recovery ($F_{6,510} = 16.4$ – 40.6 , $P < 0.001$) and slope condition ($F_{2,510} = 2.628$ – 14.5 , $P = 0.001$ – 0.073). Magnitudes of locomotor EMG activity changed similarly for each burst phase over the recovery period: the magnitude of locomotor EMG activity was close to zero in time windows W1–2 and W3–4, then began to increase, and reached the presurgery levels at time window W9–10. Magnitudes for all three burst phases were not significantly different from control values in W9–10 and W11–14 ($P < 0.050$, Fig. 6). The magnitudes of SO locomotor EMG activity before paw contact were not affected by slope ($F_{2,510} = 2.628$, $P = 0.073$), whereas activity during stance and for the entire burst was significantly greater during upslope than during downslope or level walking ($F_{2,510} = 12.4$ – 16.0 , $P < 0.001$; Fig. 6).

Durations of the three phases of SO locomotor EMG activity were also affected by the time of recovery ($F_{6,511} = 6.832$ – 8.267 , $P < 0.001$) and slope ($F_{2,510} = 153.1$ – 312.0 , $P < 0.001$; Fig. 7). In time windows W1–2 and W3–4, estimates of EMG burst durations were not reliable because of the small number of walking cycles with detectable bursts of EMG activity. Durations of all three phases of locomotor EMG activity across all slope conditions were significantly longer in W11–14 than in controls (C; Fig. 7). In fact, all three burst durations, considering all slope conditions, were significantly longer in W7–8 through W11–14 compared with controls (C; $P < 0.001$). The duration of precontact EMG activity was longer ($P < 0.001$) during downslope walking (0.082 ± 0.022 s) than during level walking (0.050 ± 0.027 s), whereas it was the shortest ($P < 0.001$) during upslope walking (0.030 ± 0.035 s). The duration of SO EMG activity during stance and that of the entire locomotor burst was longer ($P < 0.001$) during upslope walking (0.446 ± 0.090 and 0.479 ± 0.089 s, respectively) than during level walking (0.352 ± 0.057 and 0.402 ± 0.067 s). The shortest durations ($P < 0.001$)

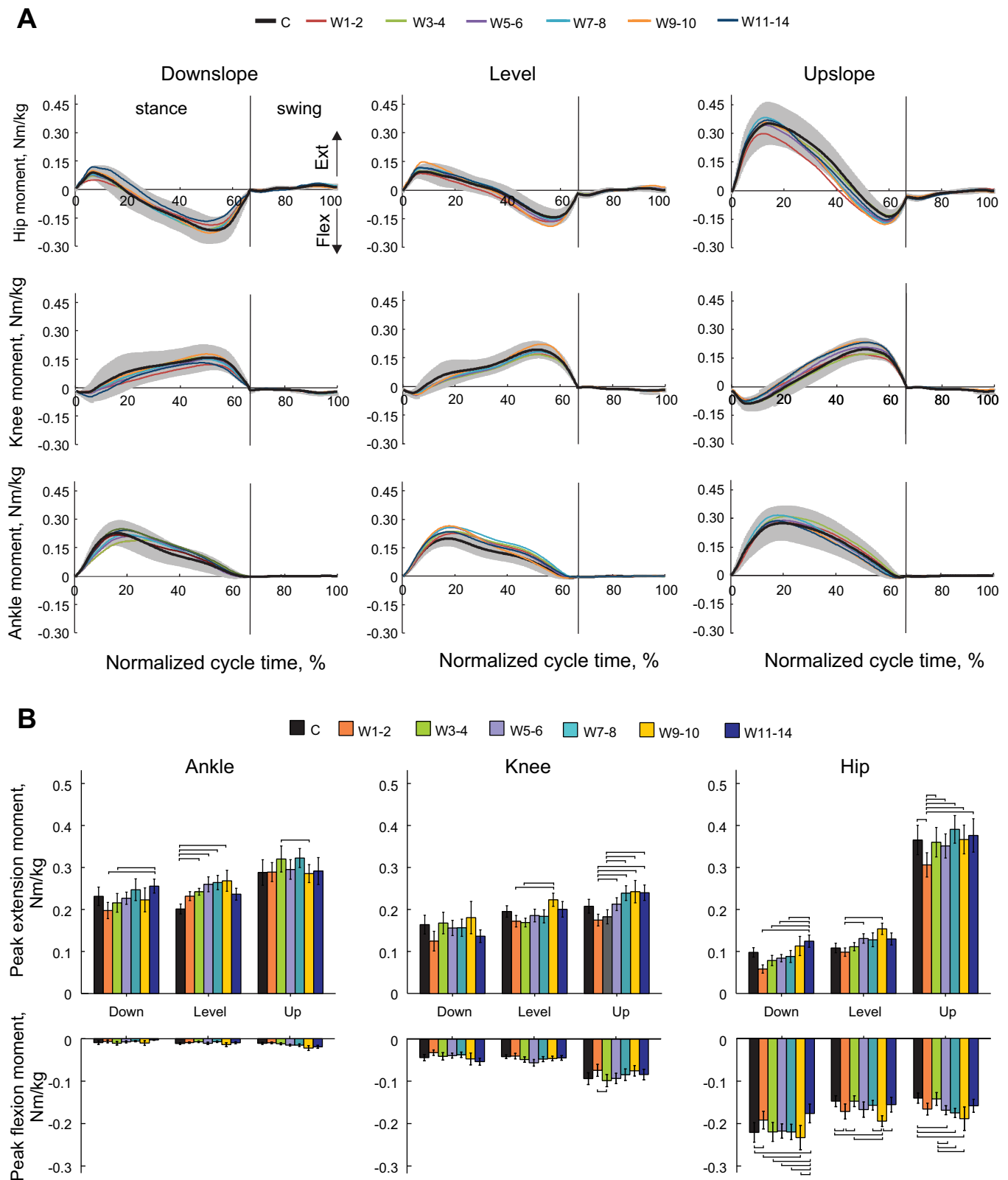


Fig. 4. Resultant moments at the hip, knee, and ankle joints during walking (abbreviations and designations of line and bar colors are the same as in Fig. 3). *A*: patterns of the joint moments during downslope, level, and upslope walking. Ext, extension; Flex, flexion. *B*: peaks of flexion (negative) and extension (positive) moments (means \pm 95% confidence interval) before Control and at 2-wk intervals in recovery after nerve injury and repair (W1–2 through W11–14). The horizontal lines between the vertical bars indicate statistical significance ($P < 0.05$).

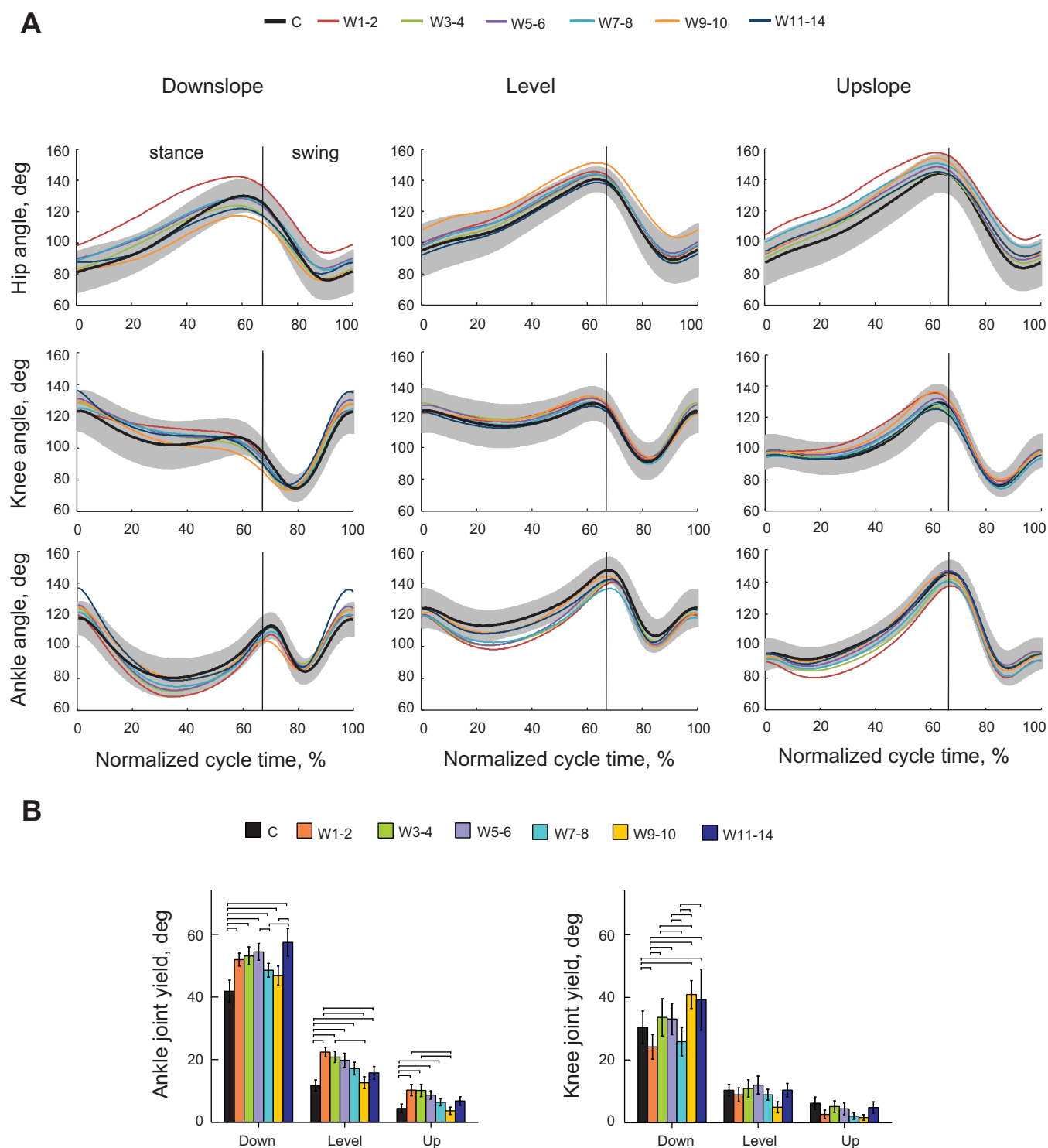


Fig. 5. Angles at the hip, knee, and ankle joints during walking (abbreviations and designations of line and bar colors are the same as in Fig. 3). *A*: patterns of joint angles during downslope, level, and upslope walking. *B*: joint yield at the ankle and knee joints during stance (means \pm 95% confidence interval) before Control and at 2-wk intervals in recovery after nerve transection and repair (W1–2 through W11–14). The horizontal lines between the vertical bars indicate statistical significance ($P < 0.05$). Yield at the hip was minimal at all slopes (1–2°) and therefore not shown here.

occurred during downslope walking (0.251 ± 0.068 and 0.333 ± 0.077 s).

Time of recovery and slope had similar effects on the three phases of locomotor EMG activity in the LG to those observed for the SO (Figs. 6 and 7). Specifically, the mean magnitude of precontact LG EMG activity was close to zero in time windows

W1–2 and W3–4 but then increased and reached presurgery levels at W9–10 through W11–14. In contrast to the SO, stance and total burst locomotor EMG activity magnitudes for the LG were not restored to presurgery levels until W11–14 (Fig. 6; $P < 0.050$). The effects of time on the mean magnitude of LG EMG activity were significant for precontact phase ($F_{6,466} =$

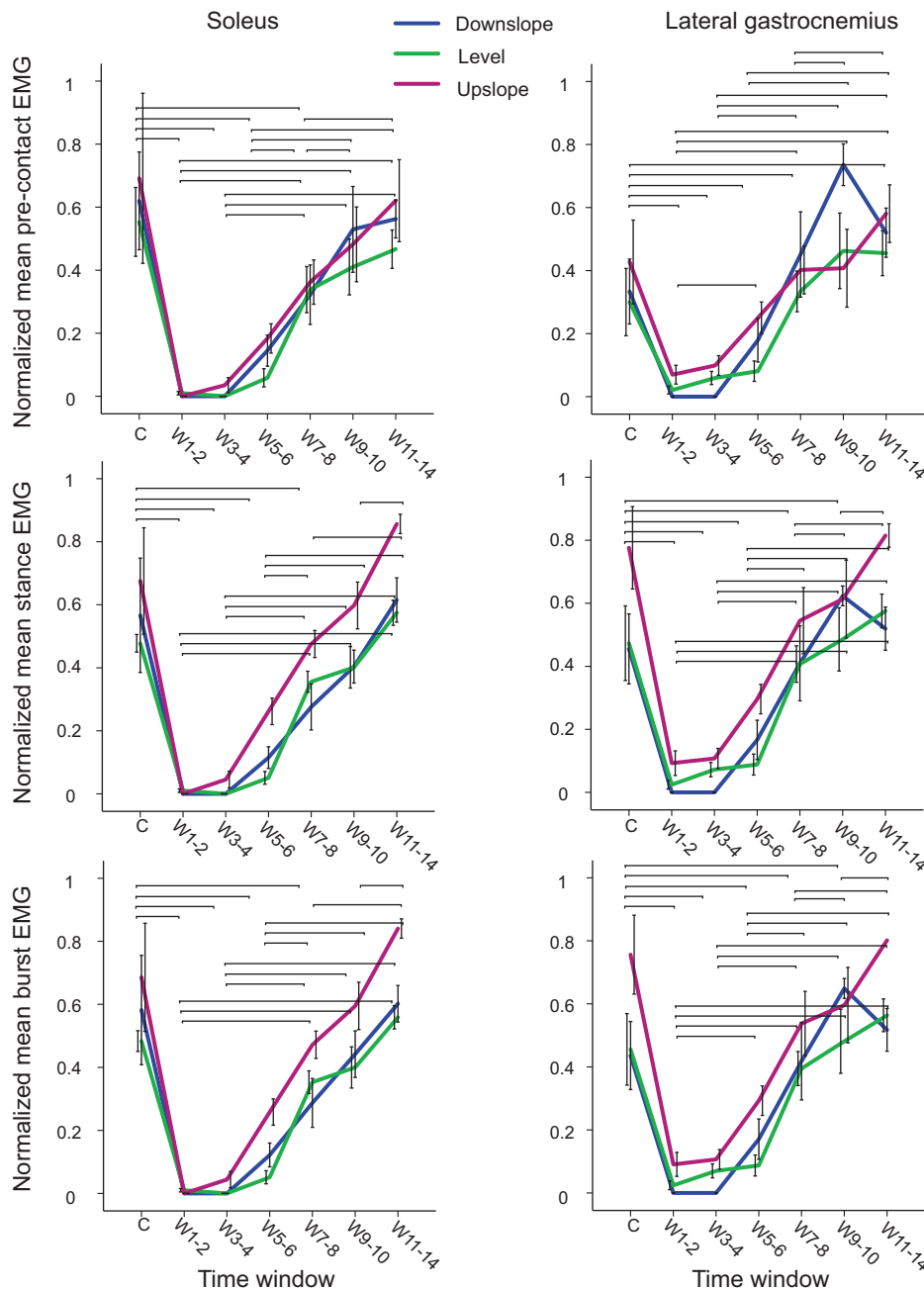


Fig. 6. Mean normalized electromyogram (EMG) activity magnitude in different phases of the EMG burst in self-reinnervated soleus and lateral gastrocnemius muscles during downslope, level, and upslope walking (means \pm 95% confidence interval). The horizontal lines indicate statistical significance between time windows across all slope conditions ($P < 0.05$). For definition of the EMG burst phases, see Fig. 1B. Definitions of time windows are the same as in Fig. 3.

30.0, $P < 0.001$), stance-related phase ($F_{6,466} = 79.1$, $P < 0.001$), and total EMG burst ($F_{6,466} = 2.043$, $P = 0.028$). Slope also had a significant effect on the LG EMG magnitude in the precontact phase ($F_{2,466} = 13.5$, $P < 0.001$), the stance-related phase ($F_{2,466} = 36.9$, $P < 0.001$), and the total locomotor LG EMG burst ($F_{2,466} = 37.2$, $P < 0.001$). The greatest LG EMG magnitude occurred during upslope walking (Fig. 6).

Duration of the LG EMG activity during the precontact phase was longer ($P < 0.001$), across all time windows, during downslope walking (0.076 ± 0.027 s) than during level walking (0.052 ± 0.029 s); the shortest duration of the precontact phase occurred in upslope walking (0.033 ± 0.023 s). The effect of slope on the duration of precontact EMG activity was significant ($F_{2,466} = 103.7$, $P < 0.001$). The durations of LG EMG activity during stance were affected by time of recovery

and slope in a manner similar to their effect on total burst durations. Both phases were significantly longer in W1–2, W3–4, W7–8, and W9–10 compared with control (C), and both were not significantly different from control (C) in W11–14 ($P < 0.050$; Fig. 7).

In summary, the results demonstrated that EMG activity of the self-reinnervated muscles reliably appeared in W5–6 showing significant increases in magnitude between W5–6 and W7–8 ($P < 0.050$). EMG magnitudes and durations generally recovered by W11–14. Precontact, stance, and total burst magnitudes reached values statistically similar to control by W9–10 for the SO muscle while the LG muscle demonstrated a more delayed pattern of recovery not reaching control values until W11–14 ($P < 0.050$). EMG magnitudes were generally greater during upslope walking except for the precontact phase,

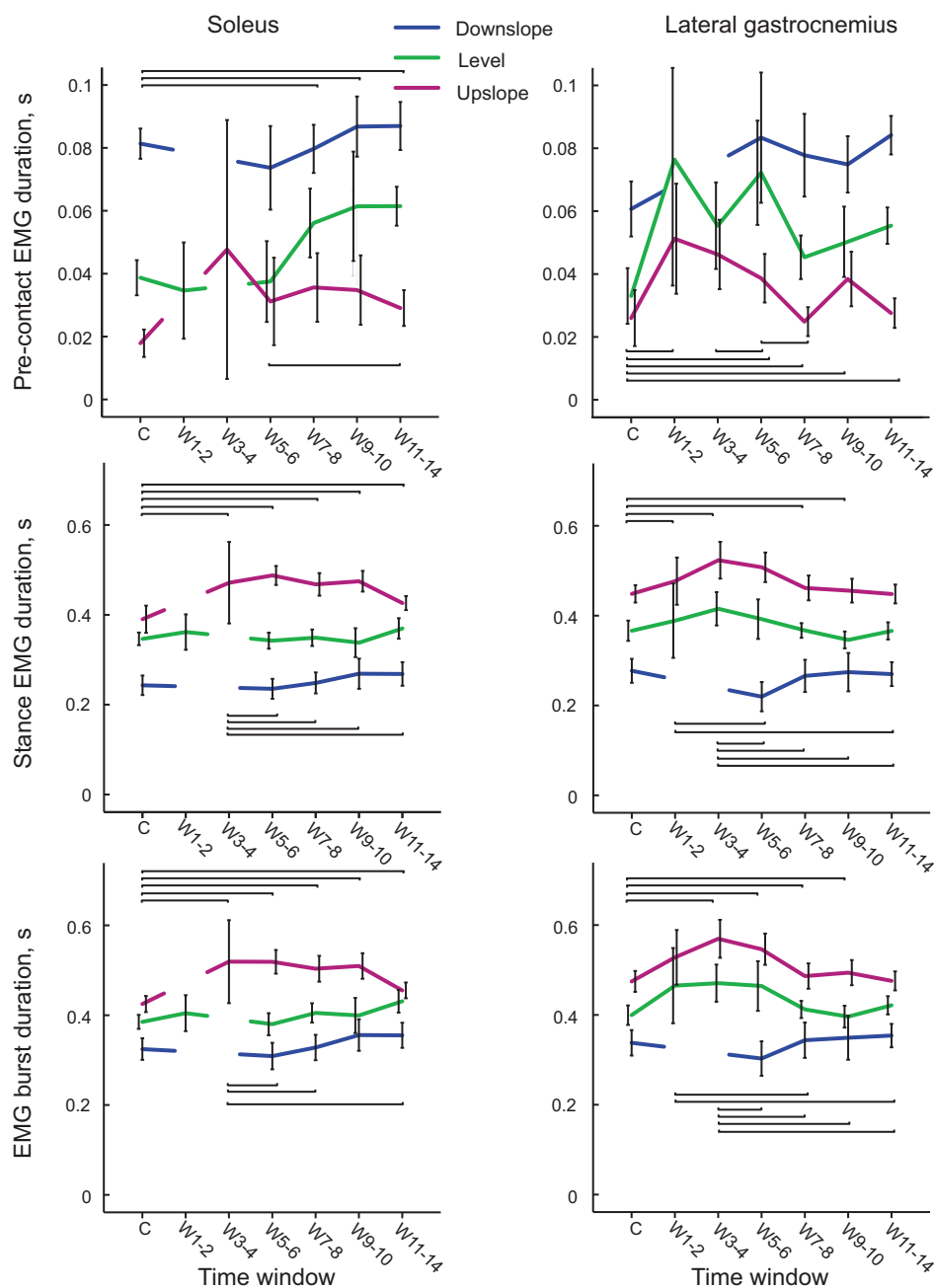


Fig. 7. Mean time duration of electromyogram (EMG) burst phases in self-reinnervated soleus and lateral gastrocnemius muscles during downslope, level, and upslope walking (means \pm 95% confidence interval). The horizontal lines indicate statistical significance between time windows across all slope conditions ($P < 0.05$). For definition of the EMG burst phases, see Fig. 1B. Definitions of time windows are the same as in Fig. 3.

in which there was no slope effect for the SO muscle. Effects of slope on EMG phase durations were similar across times of recovery but different between the precontact phase and stance-related phases. EMG activity durations in the precontact phase were longest for downslope walking and shortest for upslope walking; EMG durations in the stance-related phases were longest for upslope and shortest for downslope walking.

EMG activity of intact synergists MG and PL. The mean magnitudes of MG EMG activity during each phase of the EMG activity burst (Fig. 1B) were significantly influenced by time of recovery ($F_{6,851} = 7.7\text{--}23.5$, $P < 0.001$) and slope walking condition ($F_{2,851} = 192.3\text{--}793.0$, $P < 0.001$). The mean magnitudes of precontact MG EMG activity across all slopes were not significantly different from control (C) until W5–6 ($P = 0.005$) and again at W9–10 ($P = 0.004$), most

notably during upslope walking when magnitudes were significantly greater than control during time windows W5–6 through W9–10 ($P < 0.005$). Magnitudes in W5–6 were also significantly greater than the magnitude at W3–4 ($P < 0.004$). Precontact magnitudes at W11–14 were significantly lower compared with W9–10 ($P < 0.048$) and were not different from the presurgery control levels ($P = 1.000$; see Fig. 8). In addition, the mean magnitude of precontact MG EMG activity, across all time windows, was significantly greater during upslope than during level or downslope walking ($P < 0.001$); there was no difference in these magnitudes between level and downslope conditions.

Mean magnitudes of MG EMG activity during stance and total burst were significantly greater compared with presurgery control levels starting with time window W1–2, continuing to increase to

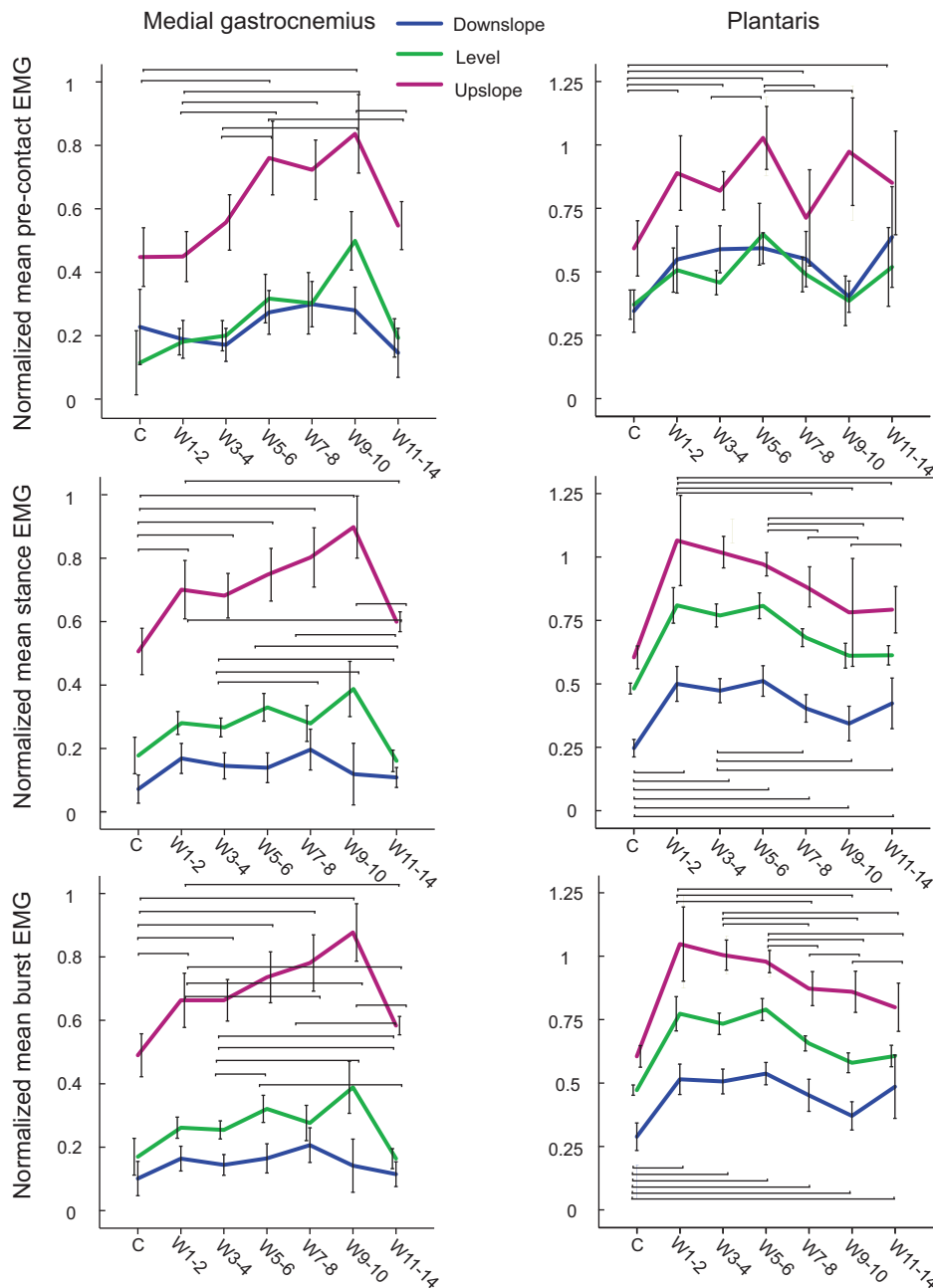


Fig. 8. Mean normalized electromyogram (EMG) magnitude in different phases of the EMG burst in intact medial gastrocnemius and plantaris muscles during downslope, level, and upslope walking (means \pm 95% confidence interval). The horizontal lines indicate statistical significance between time windows across all slope conditions ($P < 0.05$). For definition of the EMG burst phases, see Fig. 1B. Definitions of time windows are the same as in Fig. 3.

a peak value at W9–10 ($P < 0.001$). Magnitudes for stance and total burst at W11–14 were significantly less than magnitudes at W9–10 ($P < 0.001$) and not significantly different from control ($P = 1.000$). In all time windows, stance and total burst mean MG EMG activities were higher in upslope than in level walking ($P < 0.001$) and lower in downslope than in level walking except for time windows C, W7–8, and W11–14 ($P = 0.052$ – 0.473).

The duration of precontact MG EMG bursts in downslope and level walking was significantly influenced by recovery time ($F_{6,850} = 14.4$, $P < 0.001$ and $F_{6,850} = 12.2$, $P < 0.001$, respectively). For downslope and level conditions, precontact durations increased above the control values at W1–2 through W3–4 and returned to presurgery levels at W5–6 ($P = 0.050$; Fig. 9). There was no significant effect of recovery time on the duration of the precontact EMG burst in upslope walking ($F_{6,850} = 1.299$, $P = 0.255$). Slope had a significant effect on

precontact duration ($F_{2,850} = 265.1$, $P < 0.001$). When averaged across time windows, precontact durations for downslope walking were significantly ($P < 0.001$) longer (0.090 ± 0.021 s) than both level (0.060 ± 0.025 s) and upslope conditions (0.049 ± 0.017 s); precontact durations in the upslope condition were significantly shorter than in level walking ($P < 0.001$).

Stance and total burst durations of MG EMG activity were affected by time of recovery ($F_{6,851} = 2.394$, $P = 0.027$ and $F_{6,851} = 4.292$, $P < 0.001$, respectively) and slope ($F_{2,851} = 880.6$, $P < 0.001$ and $F_{2,851} = 573.0$, $P < 0.001$, respectively). Stance MG EMG durations across all slopes remained at control levels until W7–8 and significantly increased during W7–8 and W9–10 returning to control values at W11–14 ($P < 0.050$). In contrast, total burst durations were significantly higher than controls in W1–2 and W3–4 and again in W7–8

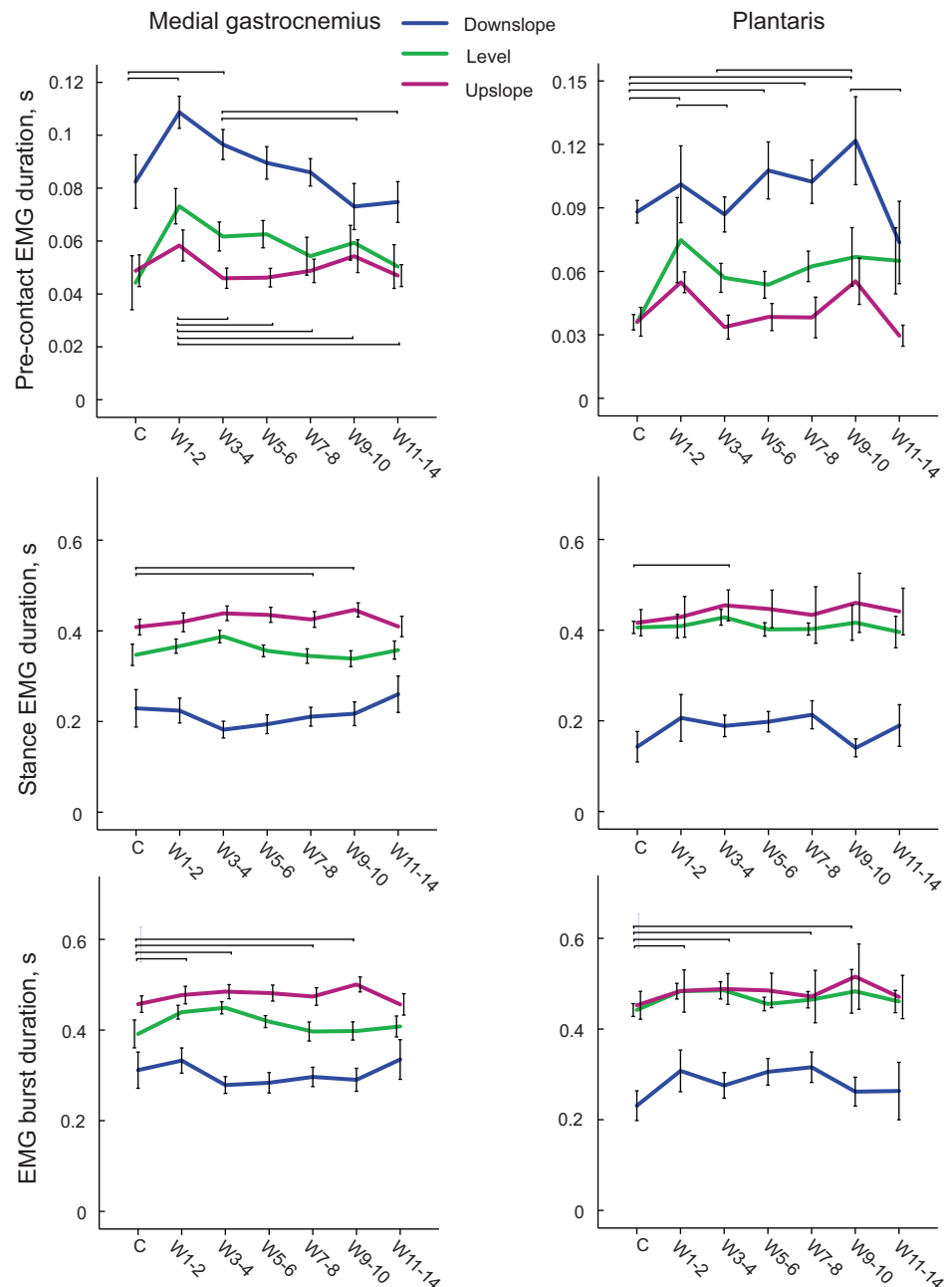


Fig. 9. Mean time duration of electromyogram (EMG) burst phases in intact medial gastrocnemius and plantaris muscles during downslope, level, and upslope walking (means \pm 95% confidence interval). The horizontal lines indicate statistical significance between time windows across all slope conditions ($P < 0.05$). For definition of the EMG burst phases, see Fig. 1B. Definitions of time windows are the same as in Fig. 3.

and W9–10 returning to the presurgery levels at W11–14 ($P = 0.030$; Fig. 9). Burst durations for stance and total burst averaged over the time windows were longer for upslope walking (0.426 ± 0.066 and 0.475 ± 0.067 s, respectively) than for level walking (0.360 ± 0.058 and 0.420 ± 0.065 s, respectively); the phase durations for downslope (0.212 ± 0.074 and 0.301 ± 0.075 s, respectively) were shorter than for level walking.

EMG magnitudes of intact synergist MG generally increased during the recovery period with peak activity in W9–10. By the end of the recovery period, EMG magnitudes returned to control values. EMG magnitudes were greater during upslope walking and smaller during downslope walking, except for precontact EMG activity, which did not differ between level and downslope walking. Durations of the precontact EMG activity demonstrated greater changes over the recovery period

than durations of the stance or whole burst EMG activity. The duration of precontact EMG was longer during downslope walking than during level or upslope walking. The durations of stance and total burst EMG activity were longest during upslope walking and shortest during downslope walking.

Changes in mean activity magnitudes in the PL were significantly affected by time ($F_{6,236} = 14.859-75.1$, $P < 0.001$) and slope ($F_{2,236} = 94.3-415.5$, $P < 0.001$, Fig. 8) in all three phases of the EMG burst. Precontact magnitudes were significantly higher than controls for almost the entire recovery period ($P < 0.001$). These significant differences were most notable during upslope walking in W1–2 through W5–6 and again in W9–10 through W11–14 and during downslope walking in W3–4 through W7–8 and again in W11–14 ($P = 0.001-0.034$). In addition, W5–6 was significantly higher than W3–4 ($P = 0.019$) and W7–8 was significantly lower than W5–6 ($P = 0.006$)

across all three slope conditions (Fig. 8). Across all time windows, precontact EMG magnitudes were significantly greater during upslope walking than during level and downslope walking ($P < 0.001$); no difference in precontact magnitudes was observed between level and downslope walking ($P = 1.0$).

Patterns of change for PL EMG stance and total burst activity were very similar during the 14-wk recovery period (Fig. 8); magnitudes significantly greater than control were observed at each week and almost all slope conditions ($P < 0.050$). The single exception was the EMG stance magnitude at W9–10, which was not significantly different from control during downslope walking ($P = 0.936$). In contrast to the MG, magnitudes in stance and total burst peaked at W1–2 for the PL instead of W9–10 as was observed in the MG. Finally, stance magnitudes in W7–8 were significantly less than in W5–6 ($P < 0.008$), those in W9–10 were significantly less than in W7–8 ($P < 0.001$), and those in W11–14 were significantly less than in W9–10 ($P < 0.024$) across all slopes. The mean PL EMG magnitudes in stance and total burst were significantly affected by slope ($F_{2,237} = 397.6$, $P < 0.001$ and $F_{2,237} = 407.1$, $P < 0.001$, respectively). During upslope walking, the magnitudes were higher than during level and downslope walking at each time window ($P < 0.001$); the magnitudes during level walking were higher than during downslope walking ($P < 0.001$) except in W11–14 for the mean EMG burst magnitude ($P = 0.202$).

Durations of the three phases of the PL EMG activity were affected by both the time and slope. The effect of time was significant for the precontact phase in downslope and level walking ($F_{6,236} = 5.568$ – 9.485 , $P < 0.001$) and for the stance-related and whole burst activity duration in downslope walking ($F_{6,237} = 2.844$ – 3.499 , $P = 0.002$ – 0.011 ; Fig. 9). In those cases, phase durations of EMG activity were longer at W5–6, W7–8, and/or W9–10 compared with control. Slope had different effects on precontact phase durations compared with the stance-related and whole burst phase durations. During downslope walking, durations of precontact PL EMG activity, averaged over all time windows (0.098 ± 0.021 s), were longer than during level (0.058 ± 0.024 s) or upslope walking (0.038 ± 0.013 s). The effect of slope on the duration of the precontact phase was significant for each time window ($F_{2,236} = 10.6$ – 66.7 , $P < 0.001$). Slope also significantly influenced stance and total burst durations ($F_{2,237} = 291.7$ and $F_{2,237} = 448.1$, $P < 0.001$); that is, the duration of these phases was shorter for downslope walking than for level and upslope walking (Fig. 9). For example, burst durations for downslope, level, and upslope walking were 0.281 ± 0.055 , 0.468 ± 0.040 , and 0.478 ± 0.069 s, respectively.

In summary, EMG activity of intact synergists MG and PL, while showing significantly greater magnitudes compared with control values for almost all slope conditions and weeks, demonstrated different patterns of response during the 14-wk period of recovery. The MG muscle showed a gradual rise in magnitude for each of the three EMG bursts reaching peak values during stance and total burst at W9–10 during level and upslope walking. Each of the three bursts then returned to values not significantly different from control in W11–14 ($P < 0.050$). The PL muscle, on the other hand, peaked immediately during W1–2 for each of the three EMG bursts analyzed, proceeding to slowly decline at W3–4 but at levels significantly greater than controls for the remainder of recovery ($P <$

0.050). EMG magnitudes for both muscles were greater during upslope walking and smaller during downslope walking, except for precontact EMG activity, which did not differ between level and downslope walking. Durations of the precontact EMG activity demonstrated greater changes over the recovery period than durations of the stance or whole burst EMG activity. The duration of precontact EMG was longer during downslope walking than during level or upslope walking. The durations of stance and total burst EMG activity were longest during upslope walking and shortest during downslope walking.

DISCUSSION

The goal of this study was to characterize the changes in EMG activity of self-reinnervated SO and LG muscles and their intact synergists MG and PL, as well as changes in locomotor mechanics during the initial 14-wk period of compensation and subsequent recovery from surgical transection and repair of the SO and LG nerves. The obtained results regarding magnitudes of locomotor EMG activity were consistent with the findings of Gordon and Stein (1982) that 1) the time required to form synapses between the regenerating axons and motor endplate is ~35 days (the middle of our time window W5–6) and 2) recovery of preoperative muscle maximum tension has a time constant of 1–2 mo (between our time windows W5–6 and W9–10).

In the following, we will discuss primarily the unique aspects of the present data, i.e., locomotor EMG and mechanics changes in time windows W5–6 through W9–10, i.e., the early reinnervation period (Gordon and Stein 1982).

Early Recovery: Compensatory Response

By separating the early recovery period, i.e., the denervated state of the SO and LG (weeks 1–4), into two 2-wk periods (W1–2 and W3–4) a number of significant changes in motor output compared with control values (W1) were highlighted. These changes were generally consistent with previous reports regarding the compensatory locomotor adjustments that occur immediately after nerve transection and repair to preserve effective gait in the short term (Maas et al. 2010; Pearson et al. 1999; Prilutsky et al. 2011) and support the notion that compensatory behavior should be addressed early in planning rehabilitation schedules to avoid permanent deleterious effects on long-term recovery (Amado et al. 2011; Scholz et al. 2009).

During a span of 4 wk after transection and repair of the nerves innervating the SO and LG muscles, significant changes from control values (C) were observed in time windows W1–2 and W3–4. Adding a second time point in early recovery allowed for a more detailed analysis than previously reported (Maas et al. 2010; Prilutsky et al. 2011). Ground reaction forces (Fig. 3), joint yield at both the ankle and knee affecting each of the four muscles studied (Fig. 5), and magnitudes and durations of locomotor EMG activity of the intact MG and PL muscles changed between W1–2 and W3–4 and between each time point and control values (Figs. 8 and 9).

Significant increases in EMG magnitude and, to a lesser extent, in duration observed at all slope conditions in the intact MG and PL during stance and total burst (Figs. 8 and 9) were concurrent with significant increases in the ankle yield (Fig. 5) and absolute lengthening imposed on the muscle-tendon unit of

all four ankle extensors during the stance phase in the first 4 wk after SO-LG nerve transection and repair (W1–2 and W3–4; Maas et al. 2010; Mehta et al. 2015; Pearson and Misiaszek 2000; Pearson et al. 1999; Prilutsky et al. 2011). The increased MG and PL EMG activity has been considered a potential compensatory mechanism, mediated by central drive and muscle length- and force-dependent afferent pathways, that sustains joint moment magnitudes at the ankle and knee and provides sufficient propulsion for walking (Donelan et al. 2009; Gritsenko et al. 2001; Maas et al. 2010; Pearson et al. 1999; Prilutsky et al. 2011).

A unique aspect of our data, however, lies in the seemingly different compensatory changes in EMG activity between the MG and PL during time windows W1–2 and W3–4. Increased magnitude of PL EMG activity was observed during both precontact and stance (Fig. 8), indicating a contribution of central drive (Gritsenko et al. 2001) as well as load- and muscle stretch-related sensory feedback, respectively. In contrast to results of Gritsenko et al. (2001), no significant increase in MG EMG activity was found during the precontact phase in either W1–2 or W3–4 (Fig. 8), although the precontact MG EMG duration did become longer (Fig. 9), as reported previously (Gritsenko et al. 2001). On the other hand, the stance MG EMG magnitude increased in W1–2 and W3–4, indicating that stance-related sensory feedback, including stretch reflex from intact ankle extensors, contributed to this increase, as suggested previously by Pearson and colleagues (Pearson and Misiaszek 2000; Pearson et al. 1999). The discrepancy in changes in the MG EMG magnitude during the precontact and stance phases with the study of Gritsenko et al. (2001) may be related to the fact that only two major ankle extensors were denervated during the first month after surgery in our study as opposed to three muscles in the study of Gritsenko et al.

As mentioned above, the observed enhanced magnitude of MG EMG activity during stance likely resulted from increased gains in cutaneous and muscle stance-related reflex responses with little, if any, contribution of central drive. This conclusion is supported by the known autogenic and heterogenic excitation of MG during locomotion mediated by Ia-afferents (Guertin et al. 1995; Ross and Nichols 2009). Note that exaggerated ankle yield (Fig. 5) leads to a greater elongation of MG muscle fascicles in early stance soon after denervation of its synergists (Maas et al. 2010). In addition, the heterogenic excitation of MG through Ib-afferents (Conway et al. 1987; Donelan et al. 2009; Guertin et al. 1995; although see Ross and Nichols 2009) and excitation of hindlimb extensors via paw pad cutaneous afferents (Duysens and Pearson 1976; Guertin et al. 1995) could contribute to the observed increase in MG EMG magnitude during stance. Given a clear dependence of the magnitude of EMG activity in the MG and PL on the slope conditions (Figs. 8 and 9), the contribution of load-dependent sensory feedback to compensatory changes in MG and PL EMG seems dominant. Indeed, the MG and PL EMG magnitudes were largest during upslope walking and smallest during downslope walking (Fig. 8), whereas peak normal (Fig. 3) and peak resultant ground reaction forces [Fig. 5 given by Gregor et al. (2006)] are the highest and ankle yield (Fig. 5B) is the lowest during upslope walking. Load-dependent paw pad cutaneous (Duysens and Pearson 1976; Guertin et al. 1995) and muscle group Ib afferents (af Klint et al. 2010; Bachmann et al. 2008; Conway et al. 1987; Pearson and Collins 1993) have been

implicated in enhancing activity of hindlimb extensors during the extensor (stance) phase of fictive and intact normal locomotion in cats and leg extensors in normal walking in humans.

Midrecovery Period: Early Reinnervation Period

During the first 4 wk of recovery, W1–2 through W3–4, the neuromuscular system relied, in part, on functional compensations by intact synergists, MG and PL, to achieve the overall goal of effective gait. During the subsequent 4 wk of recovery, i.e., W5–6 through W7–8, the resources available to the nervous system increase as the reinnervation process moves forward and denervated muscles begin to regain their ability to contribute more to the ankle and knee moments during gait. The “midrecovery period,” or “early reinnervation period” (Gordon and Stein 1982), is the period when the neuromuscular system begins to adapt to the permanent loss of the stretch reflex in the SO and LG as a result of surgical nerve transection and repair (Alvarez et al. 2011; Cope and Clark 1993; Cope et al. 1994; Haftel et al. 2005) and begins to relearn how to use previously relied upon resources, i.e., the SO and LG muscles, as they become available through the self-reinnervation process (Sanes and Lichtman 1999; see also Fig. 2).

Recovery of self-reinnervated SO and LG. The time course of recovery of reinnervated motor units after nerve transection and repair has been discussed in the literature and shows that the delay before actual recovery, i.e., the time it takes for regenerating axons to secure peripheral connections in the cat triceps surae, averages 35 days (Gordon and Stein 1982, their Fig. 3). After this initial delay, complete recovery of preoperative muscle force has a time constant of 1–2 mo (Gordon and Stein 1982). The formation of synapses with fully differentiated muscle fibers is the most important aspect of the reinnervation process and appears to be independent of the actual type of target fibers (Sanes and Lichtman 1999). Once a connection is made (Fig. 2), transmission of impulses to reinnervated muscle fibers begins almost immediately and progresses as the presynaptic and postsynaptic membranes mature (Sanes and Lichtman 1999). Consistent with this time frame, we found that EMG activity magnitudes during stance and total burst in the SO and LG muscles (Fig. 6) significantly increase from time window W5–6 to time window W7–8, i.e., 43–56 days post-nerve transection and repair ($P < 0.001$). This was most apparent during upslope walking for total EMG burst magnitudes in both muscles and may be due, in part, to the significant increase in central drive (see higher precontact EMG magnitudes in the SO in W7–8 compared with W5–6), as well as to the preservation of the intermuscular length and force-dependent feedback in self-reinnervated muscles (Lyle et al. 2016). For example, the preserved intermuscular excitatory length feedback from self-reinnervated LG could contribute to SO EMG activity (but not vice versa) during downslope walking, during which the gastrocnemius fascicles undergo large elongations (Maas et al. 2009, 2010). The reduced inhibitory force feedback from self-reinnervated gastrocnemius heads to the ankle extensor flexor hallucis longus (Lyle et al. 2016) could increase the contribution of flexor hallucis longus to the ankle extension moment (Fig. 4). It should be noted that the preserved intermuscular force-dependent feedback was revealed in the nonlocomoting decerebrate cat during the cross-extension reflex and that the feedback was inhibitory (Lyle et al.

2016). In locomoting decerebrate preparations, however, the inhibitory force-dependent feedback pathways close, and new intermuscular excitatory force-dependent pathways open (Conway et al. 1987; Gossard et al. 1994; Guertin et al. 1995; Pearson and Collins 1993; however, see Ross and Nichols 2009). Thus this intermuscular excitatory force feedback could contribute to the increase in the SO and LG stance-related activity given that the activity magnitude was higher in upslope than in level and downslope walking (Fig. 6) and ground reaction forces were larger (Fig. 3) and muscle stretch was smaller (Maas et al. 2009, 2010) in stance of upslope walking than in level or downslope conditions.

While EMG burst magnitudes during midrecovery remained significantly lower than control for stance and total burst, the significant increase in the magnitude observed within W7–8 above previous values in W5–6 is an important finding. A functional consequence of these significant increases in EMG activity, at this point in recovery, was a significant decrease in yield at the ankle in W7–8 compared with W5–6 (Fig. 4) during downslope walking. This is an interesting observation because downslope walking challenges the length-dependent pathways more than level and upslope walking, and previous reports only compared the ankle yield magnitudes during slope walking with control values (Abelew et al. 2000; Maas et al. 2007).

The recovery of self-reinnervated skeletal muscle to a viable force-producing end organ of the nervous system and the strategies used to regain both the quality and quantity of the individual motor units have been discussed extensively in the literature (Brushart 2011; English 2005; Foehring et al. 1986a, 1986b; Gordon and Stein 1982; Gordon et al. 2004; Pantall et al. 2016; Rafuse and Gordon 1996a). Pantall et al. (2016), using a wavelet and principal component analysis, have shown that after 12 wk of recovery from SO-LG nerve transection and repair, EMG intensity increased and frequency decreased in the reinnervated SO and LG and the intact MG and PL. Synchronization of activity in muscle fibers of regenerated motor units that increased in size after muscle self-reinnervation and the additional recruitment of slower motor units were suggested as potential mechanisms for this finding. Changes in motor unit types and size, individual fiber size, and distribution of motor units within the reinnervated muscle can be related back to the nature of connections made by the regenerating motor axons and can be projected forward as a major influence on the success of the reinnervation process and any deficits observed in functional motor output during gait (Pantall et al. 2016). While the limited anatomical analyses presented here preclude establishing a precise timeline for muscle reinnervation, we believe that the parallel between our muscle histology findings (Table 2 and Fig. 2), the recovery time of the self-reinnervated muscles obtained by electrical stimulation of the proximal nerve stump (Gordon and Stein 1982), and the recovery of hindlimb functional outcomes (locomotor EMG activity and hindlimb mechanics) is not trivial and represents important findings.

Changes in synergists MG and PL. Magnitudes of MG and PL locomotor EMG activity remain significantly higher than control, and those of the reinnervated SO and LG remain significantly lower than control, for stance and total burst during the midrecovery period (Figs. 6 and 8). A significant feature of these results, however, lies in the fact that the

contribution made by the two intact extensors, MG and PL, appears to change during W5–6 and W7–8. As the self-reinnervated SO and LG begin to assume a greater role in their contribution to the ankle joint moment, the MG, after a plateau in magnitude in W1–2 and W3–4, continues to increase its contribution. In contrast, the PL, after reaching peak magnitude in W1–2, continues to decrease its contribution to the ankle moment. Specifically, stance and total burst magnitudes in the PL in W7–8 were significantly less than values in W3–4 and W5–6, while MG values in W7–8 were significantly greater than W3–4 but not significantly different from W5–6 in both stance and total burst (Figs. 6 and 8).

One potential mechanism explaining the different changes between MG and PL EMG activity may be related to the central drive to these muscles. Precontact EMG magnitudes, used as an indicator of central drive, peaked in W5–6 for the PL; that is, they were significantly greater than W3–4, W7–8, and control values (Fig. 8). In contrast, precontact magnitudes in the MG were not significantly greater than control in W1–2 and W3–4 but were in W5–6 through W9–10 (Fig. 9). Central drive appeared to be a contributing factor in W5–6 for the MG's continued increase in EMG magnitude and subsequent force contribution to the extensor moment, while the significant decrease in central drive in W7–8 for the PL may have contributed to the continued decline in EMG magnitudes and subsequent force contribution of the PL. These changes in burst magnitudes for the PL and MG were observed at all slope conditions, the most prominent observed during upslope walking, which would also potentially involve load-sensitive receptors in the paw and muscles as previously discussed (Maas et al. 2007, 2010). Additionally, the significant decrease in yield observed at the knee and a slight decrease at the ankle between W5–6 and W7–8 would result in no significant effect in muscle-tendon unit length change, minimizing the effect of length-dependent input on the increased EMG in the MG and PL as argued during early recovery (Maas et al. 2010) and further enhancing the contribution of central drive.

Late Recovery Period: Adaptation Period

The time frame for late recovery in this analysis is 9–14 wk (56–98 days, W9–10 through W11–14), a period in which the SO and LG would be expected to reach their preoperative force capabilities (Gordon and Stein 1982) and locomotor output would have little, if any, detectable deficits during level and upslope walking (Gregor et al. 2014; Maas et al. 2007; Mehta and Prilutsky 2014; Pantall et al. 2016). While our data are generally consistent with these expectations, some additional observations warrant further discussion.

First, stance and total burst EMG in the SO reached magnitudes statistically similar to control values earlier in recovery (W9–10) than did the magnitudes in the LG (Fig. 6). The functional consequence of this time difference between the two self-reinnervated muscles would be that the SO achieved a normal level of force production and subsequently its normal contribution to the ankle extension moment earlier in recovery than the LG. This time difference may be due, in part, to the nature of the self-reinnervation process within each muscle related to changes in size and type of the reinnervated motor units (Brushart 2011; Pantall et al. 2016), to the actual nonlinear time differences for the regenerating axons to reach their new targets, and to the integrity of single

motor units at that time (Gordon and Stein 1982); see Table 2. Central drive appears to have minimal, if any, effect on these differences because precontact EMG magnitudes were not significantly different from controls in either W9–10 or W11–14 for both reinnervated muscles. These data related to central drive in the SO and LG are similar to those reported by Pantall et al. (2016).

Precontact, stance, and total burst EMG magnitudes for the PL remained higher than control in W9–10 and W11–14; this was true for the MG in W9–10 alone (Fig. 8). These data for the PL are in contrast to those reported by Pantall et al. (2016, their Figs. 2 and 7) and may be due, in part, as mentioned in that report, to the differences in trials selected for data analysis based on stance time and our conservative approach here in our additional use of stance time as a covariate in our analysis. Also, the comparisons described by Pantall et al. (2016) were between control values and values 10–12 wk in recovery.

EMG magnitudes during stance and total burst were significantly higher than control in the MG during W9–10 and in the PL during W9–10 and W11–14. These increased magnitudes may be related, in part, to increased central drive in W9–10 in the MG and in W9–10 and W11–14 in the PL and to enhanced gains in the load-dependent sensory pathways. The contribution of the stretch-related sensory pathways is expected to be smaller because of the compliance of MG and PL muscle-tendon unit resulting in small or no stretch of muscle fascicles during the stance phase of level and upslope walking (Hoffer et al. 1989; Maas et al. 2009; Prilutsky et al. 1996) and because MG and PL EMG magnitudes during stance were consistently higher in level than in downslope walking despite the similar central drive to these muscles in these slope conditions (Fig. 8).

Functional and Clinical Significance

The results of this study identified three distinct periods of functional recovery after peripheral nerve transection and repair of SO and LG muscles in the cat. In the early recovery (W1–2 through W3–4), compensatory changes in EMG activity of intact synergists and hindlimb posture permitted the maintenance of nearly normal values of joint moments beginning in W3–4. Locomotor exercise interventions during this early compensation period have been reported to accelerate regeneration of motor and sensory axons, reduce the withdrawal of synapses from motoneurons in the spinal cord (Brandt et al. 2015), and optimize compensatory locomotor strategies. In the midrecovery period, or early reinnervation period (W5–6 through W7–8), regenerating axons reached target muscle fibers and began to reoccupy motor endplates. This results in the recovery of the number and size of single motor units, potential motor unit types, and total muscle mass; increasing EMG magnitudes in self-reinnervated SO and LG muscles; decreased EMG magnitudes in the PL; and increased magnitudes in the MG muscles. These changes, in turn, appeared to reduce locomotor deficits throughout the periods of compensation and recovery. Locomotor exercises during this early reinnervation period are expected to speed up the process of recovery of muscle fiber size and muscle mass and promote the use of recovering feed-forward control of self-reinnervated muscles, as well as some sensory feedback pathways (Brandt et al. 2015; Lyle et al. 2016). In the late recovery (W9–10 through W11–14), neuromuscular synapses occupied almost

100% of motor endplates, the EMG magnitude and timing of self-reinnervated muscles reached or exceeded the preinjury values, and locomotor mechanics approached patterns similar to those observed in control. Locomotor exercise during this period is expected to maintain normal function of self-reinnervated muscles and their sensorimotor control. On the basis of the current literature (Alvarez et al. 2011; Bullinger et al. 2011; Haftel et al. 2005; Lyle et al. 2016), we would not expect the recovery of stretch reflex in self-reinnervated muscles.

Absence of the stretch reflex in two major ankle extensors, SO and LG, appears to be well compensated during level and upslope walking (but not during downslope walking) by the end of our 14-wk recovery period (W11–14). Load-dependent excitatory feedback from paw pad afferents and ankle extensor Golgi tendon organs may contribute, in part, to the activity of hindlimb extensors during the stance phase of locomotion (Conway et al. 1987; Duysens and Pearson 1976; Guertin et al. 1995; Pearson and Collins 1993). Increased central drive, as described above (Fig. 8), also seems to be a contributor to extensor activity, especially noted in the PL during upslope walking. On the other hand, contributions through heterogenic Ia excitatory pathways from intact synergists MG and PL to self-reinnervated SO and LG (Eccles et al. 1957; Nichols 1994) are likely to be weak or absent during level and upslope walking because of the lack of any substantial stretch of muscle fascicles in the MG and PL during stance (Maas et al. 2009; Prilutsky et al. 1996). Intermuscular inhibitory force-dependent pathways, although preserved in self-reinnervated muscles (Lyle et al. 2016) and possibly operational during locomotion (Ross and Nichols 2009), do not appear to provide a substantial contribution to recovered SO and LG EMG magnitudes and durations during stance given the observed increase in their EMG activity from downslope to upslope walking conditions (Figs. 6 and 7), i.e., with increasing hindlimb loading. The same can be said about the potential contribution of autogenic inhibitory force-dependent feedback, although it is currently unknown whether it recovers after muscle self-reinnervation. Consistent with the idea regarding excitatory load-dependent feedback during the stance phase of locomotion is the dependence of EMG magnitude and burst duration on slope during stance in all ankle extensors (except the denervated SO and LG in W1–2 and W3–4). Slope effects on the precontact EMG activity, reflecting central drive, were smaller (Figs. 6–9). Magnitude and duration of EMG activity during stance and total burst were the greatest during upslope walking and the smallest during downslope walking, in which the normal components of ground reaction forces were the highest and lowest, respectively (Fig. 3B; see also Fig. 5 given by Gregor et al. 2006).

Somatosensory motion-dependent feedback from the contralateral hindlimb and ipsilateral and contralateral forelimbs could also contribute to compensatory central drive [the ipsilateral hindlimb central pattern generator (CPG) output] and interlimb reflex responses via the spinal commissural, diagonal, and homolateral interneuronal pathways (Frigon 2017; Jankowska 2008; Talpalar et al. 2013). The exact contributions of these inputs to the compensatory activity of self-reinnervated muscles could not be evaluated in this study, because we analyzed mechanics and muscle activity of only the affected hindlimb.

Duration of the precontact EMG phase (EMG onset time before paw contact) was, on the contrary, longer during downslope walking and shorter during upslope walking (Figs. 7 and

9). This result is consistent with the role of length-dependent feedback (Ia- and II-afferents) from hip extensor muscles in initiating the flexor-extensor phase (swing-stance) transition and with previous reports on intact cats (Gregor et al. 2006; McVea et al. 2005). Muscle-tendon unit lengths of hip extensors reach maximum values in late swing (Gregor et al. 2006) because of the large hip flexion angles in this phase of gait (Fig. 5A; see also Gregor et al. 2006; McVea et al. 2005).

Computational experiments with neuromechanical models of cat hindlimb locomotion (Ekeberg and Pearson 2005; Markin et al. 2016; Yakovenko et al. 2004) have provided additional evidence on the contribution of different proprioceptive inputs to muscle activity and stability of walking, which is consistent with the above discussion. Ekeberg and Pearson (2005) have demonstrated a greater contribution to the maintenance of stable walking of the ankle extensor group Ib (load-dependent) afferent input than the input from hip flexor group Ia and II (length-dependent) afferents. The stabilizing role of Ib input from ankle extensors during walking was confirmed in computer simulations of Markin et al. (2016), who also demonstrated the stabilizing role of Ia input from hip extensor muscles. Specifically, removal of excitatory Ib force feedback from ankle extensors or Ia length feedback from hip extensors resulted in collapse of the walking model, whereas after removal of Ia length feedback from ankle extensors, the model continued stable locomotion (Markin et al. (2016, their Figs. 2.15 and 2.18). The latter result on the role of stretch reflex of ankle extensors during walking is consistent with results of the computational study of Yakovenko et al. (2004), who concluded that if the central drive to muscles is adequate, the contribution of stretch reflex is less significant. Note that sensory pathways from ankle extensor group Ib afferents and hip extensor group Ia and II afferents (but not ankle extensor group Ia afferents) have been suggested to access circuitry of the CPG (Gossard et al. 1994; McCrea and Rybak 2007; Pearson 2008). On the basis of results of this study and computational experiments (Markin et al. 2016; Yakovenko et al. 2004) it could be proposed that removal of Ia stretch-sensitive feedback as a result of nerve transection and repair in pathways accessing the central pattern generator (e.g., from hip muscles) would lead to more severe locomotor deficits than we observed in the present study. For example, length-sensitive group Ia and II afferent input from hip muscles has been shown to entrain and/or reset ongoing CPG activity (Kriellaars et al. 1994; Lam and Pearson 2001; Perreault et al. 1995; Stecina et al. 2005). Thus self-reinnervation of hip muscles will likely diminish important Ia sensory input to CPG and disturb regulation of locomotor phase transitions.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

R.J.G., H.M., A.W.E., and B.I.P. conceived and designed research; R.J.G., H.M., M.A.B., A.W.E., and B.I.P. performed experiments; R.J.G., H.M., M.A.B., A.O., A.W.E., and B.I.P. analyzed data; R.J.G., H.M., M.A.B., A.W.E., and B.I.P. interpreted results of experiments; A.O. and B.I.P. prepared figures; R.J.G. and B.I.P. drafted manuscript; R.J.G., H.M., M.A.B., A.O., A.W.E., and B.I.P. edited and revised manuscript; R.J.G., H.M., M.A.B., A.O., A.W.E., and B.I.P. approved final version of manuscript.

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